Translation of PCT/DE 96/01269
filed: 12.07.1996

P 2941 WO US
V/Ni

METHOD FOR DETERMINING THE EFFECTIVENESS AND TOLERANCE OF A XENOGENIC SUBSTANCE ADMINISTERED TO AN ORGANISM

BACKGROUND OF THE INVENTION

Prior Art

The invention concerns a method for the determination of the activity of immune cells in dependence on an active substance, ~~in accordance with the precharacterizing part of the main claim~~.

Determination of the tolerance and effectiveness of pharmaceutical products such as homoeopathic, biological, natural and chemical compounds and compound mixtures for the patient being treated is decisive for successful therapy and treatment of a disease. The reaction of the patient to the pharmaceutical product is normally studied subsequent to administration.

A number of methods are known in the art for investigating the activity of immune cells with regard to various cells.

A conventional method (In: Naturwissenschaften 76, page 530 ff., 1989), the influence of the mistletoe plant (Viscum album) on the activity of immune cells is investigated with regard to cancer cells. Towards this end, cancer cells are marked with radioactive tritium and mixed with immune cells and mistletoe extract. The remaining amount of radioactivity of the mixture is determined after a certain period of time to provide information about the number of cancer cells destroyed by the immune cells. This method has the disadvantage that radioactive isotopes are necessary, that the precision depends on the number of cells investigated, and that the method is difficult and time consuming to carry out.

In another conventional investigation (In: Blood, Vol. 84, No. 10, November 1994, pages 3440 - 3346), the chemical sensitivity of B-lymphocytes is determined using the MTT-analysis procedure. Towards this end, the cytotoxicity of ambozile chloride (CLB) is compared with that of fludarabine, DNA topoisomerase 1 inhibitors, and other compounds. The B-lymphocytes thereby investigated come from patients suffering from B-CLL (chronic lymphocytic leukaemia). This investigation strives to develop a general prediction concerning the effectiveness of the compounds utilized and investigated for a group of patients suffering from a particular disease.

In the conventional method characterizing the instant invention (In: Cancer Immunology Immunotherapy, pages 393-398, 1992), the influence of intrinsic body compounds (Interferon γ, Tumor-Nectrosis-Factor-α) on cytostatica and cytotoxicity is investigated in human tissue cells (U 937). This method attempts to effect a general statement concerning the effectiveness of the compounds being investigated on certain types of tissue cells. The method concerns confirmation of a particular expected effectiveness.

~~The Invention and its Advantages~~.

In contrast thereto, ~~the method in accordance with the invention having the features in the characterizing portion of the main claim has the~~ advantage that the tolerance and effectiveness of xenogenic pharmaceutical products on a human being or on individual animals can be determined. Towards this end, the scientifically developed conventional method is used in accordance with the invention for a new industrial application.

The goal of the conventional investigation characterizing the invention is to determine the effect of individual body compounds on immune cells and target cells, which could be infected cells, cancer cells or other cells. The primary aspect thereby is to develop a general statement concerning whether or not a particular compound has a certain

relationship to immune cells and the immune system of particular living organisms.

The new application in accordance with the invention of the modified procedure is not, in contrast thereto, intended to develop a general overall statement concerning the effect of certain individual body compounds. Rather, the individual tolerance and effectiveness of a pharmaceutical product not naturally occurring in the body is determined for a single patient. The results of the method provide information concerning the possible success of treatment. In this manner it is possible to determine, with the assistance of the method in accordance with the invention, whether and to what extent an individual patient reacts to a pharmaceutical product prior to administration of this pharmaceutical product. A plurality of similar compounds can also be tested to determine the one to which a particular patient best responds. For investigations of this type, blood is extracted from the patient from which the immune cells are isolated. These cells are then mixed with special target cells, e.g. with virus-infected cells, with cancer cells or with normal cells (autogenic, allogenic or xenogenic cells) and with the pharmaceutical product.

Without doubt, there has been a long felt need for a simple and economical method for determining and predicting whether or not and to what extent a patient would react to a pharmaceutical product and the chances for successfull treatment prior to the administration of the pharmaceutical product. The method in accordance with the invention satisfies this need.

Although the method of prior art categorizing the instant invention has been known in the art since 1992 and although the activity analysis based on tetrazolium salt MTT for carrying out the method has been known in the art and used for similar purposes in scientific investigations since at least 1988 (see Cancer Research 48; 589-601, 1988), this conventional method has only been used for analytic scientific applications up to the point of time of the instant invention.

An additional advantage of the method in accordance with the invention is that extremely expensive and/or risky pharmaceutical products having side-effects can be tested for effectiveness and tolerance to patients prior to administration thereof. Precisely such compounds often require proof of effectiveness of the pharmaceutical product for insurance purposes in order to recover costs for the pharmaceutical product.

An additional advantage of the invention is that the method can be carried out simultaneously with or, in the invent of undesirable effects, in series using different xenogenic pharmaceutical products and/or pharmaceutical product mixtures. Proceding in this manner has the advantage that the optimal effectiveness and tolerance of possible alternative xenogenic pharmaceutical products from which a selection is to be made can be determined.

In accordance with an advantageous embodiment of the invention, homoeopathic compounds and compound mixtures are utilized as the xenogenic pharmaceutical product. In particular, for homoeopathy, in which very low doses of compounds which, in healthy individuals at higher doses create a similar disease pattern, are administered to treat diseases, it is reasonable to determine the effectiveness and tolerance of the compound for the patient prior to administrating same. In addition, the method has the invention that the effectiveness and tolerance can be tested at doses higher than those used on the patient.

In accordance with an additional advantageous embodiment of the invention, cancer cells are utilized as target cells. In this manner, the effect of a pharmaceutical product can be investigated, in particular with cancer patients.

In accordance with an additional advantageous embodiment of the invention, virus-infected cells are utilized as target cells.

In accordance with an additional advantageous embodiment of the invention, normal cells (allogenic, autogenic or xenogenic cells) are utilized as target cells.

In accordance with an additional advantageous embodiment of the invention, tetrazolium salts are utilized as coloring agents.

In accordance with an additional advantageous embodiment of the invention, the tetrazolium salt MTT is utilized as the substrate. This substrate is a yellow tetrazolium salt. The dehydrogenase in the active mitochondria of living cells converts this yellow salt into a blue formazan crystal. This transformation only occurs in living cells and has differing strength in immune cells and target cells. With the assistance of a spectrometer, the color of the sample can be investigated which, in turn, provides information concerning the number of target cells which have been destroyed by the immune cells.

In accordance with an additional advantageous embodiment of the invention, the tetrazolium salt XTT is utilized as the substrate. This substrate provides more precise measurement results for specific applications.

Further advantages and advantageous embodiments of the invention can be extracted from the claims.

All of the features represented in the description and the subsequent claims can be significant to the invention individually or collectively in arbitrary combination.

A I CLAIM: